Patent
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
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HIGASHIYAMA et al	)	Group Art Unit: 1617
	)	
Application No.: 09/254,152	)	Examiner: S. Wang
	)	
Filed: February 26, 1999	)	Confirmation No.: 6530
	)	
For: PROCESS FOR PRODUCING UNSATURATED FATTY ACID-CONTAINING OILS	)	

DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Kenichi Higashiyama, hereby declare as follows:

1. I am a co-inventor for the above-identified application.

2. The following experiments were performed either by me, or under my direct supervision and control.

3. Experiment No. 1

Microorganism:

Mortierella alpina 1S-4 was used for this experiment. This strain is the same as that used in reference [1] (Shimizu et al, *Lipid* 27:481-83 (1992)), cited by the Examiner in the Official Action dated July 16, 2002.

Material and Methods

The fungus *M. alpina* 1S-4 was cultivated in a medium (6 L, pH 6.0) containing 2% glucose and 1% yeast extract in a 10-L fermentor for 7 days at 28°C with agitation speed of 300 rpm and aeration rate of 6 L/min. These conditions were similar to those used in Reference [1], however, a fermentor with aeration was used in this experiment.

The culture broth was harvested at 3 and 7 days of cultivation, and then analyzed following the method described in the reference [1] (Shimizu et al, 1992).

Results

It was confirmed that 24,25-methylenecholest-5-en-3β-ol compositional ratio is higher in the aeration culture using the fermentor than in the shake culture using a flask even though the same culture medium was used.

Table 1. Culture conditions and results of Experiment 1

Culture Conditions			
Experiment	Experiment No. 1		Reference [1]
Cultivation method	Aeration culture in a fermentor		Shake culture in a flask
Cultivation time	at 3 days of cultivation	at 7 days of cultivation	6-8 days
Results			
24,25-methylenecholest-5-en-3β-ol compositional ratio (%) (=A)	40.0	45.5	21.7*
Desmosterol compositional ratio (%) (=B)	31.0	34.2	58.6*
A/B	1.29	1.33	0.37

*Calculated from the data of content in Table 1 of the reference [1] (Shimizu et al, 1992)

4. As can be seen from the most right column, the method of Reference 1 provides a low ratio of 24,25-methylenecholest-5-en-3β-ol. On the other hand, as can be seen from the central columns, when the same method is repeated in a fermentor with aeration, the ratio of 24,25-methylenecholest-5-en-3β-ol is high.

5. It is my opinion that the 1.29 and 1.33 ratio of 24,25-methylenecholest-5-en-3β-ol is significantly higher than the claimed ratio of 1.20 or less, as recited in the instantly claimed invention. This A/B ratio is also significantly higher than the ratios of 0.9 or lower and 0.6 and lower.

6. Experiment No. 2

Microorganism

Mortierella alpina 1S-4 was used for this experiment. This strain is the same one as that used in the culture of Figure 3 in reference [2] (Y. Shinmen et al, *Appl. Microbiol. Biotechnol.* 31:11-16 (1989)).

Materials and Methods

The fungus *M. alpina* 1S-4 was cultivated in a medium (6 L, pH 6.3) containing 2% glucose, 1% yeast extract and 0.2% soybean oil in a 10-L fermentor for 7 days at 28°C with agitation speed of 300 rpm and aeration rate of 6 L/min. Glucose was added periodically to the medium for prevention of glucose depletion.

The culture broth was harvested at 7 days of cultivation, and then analyzed. Determination of arachidonic acid and sterol composition were carried out following the methods described in reference [2] (Shinmen et al, 1989) and reference [1] (Shimizu et al, 1992), respectively.

Results

Experiment No. 2 was carried out under similar conditions to that of Figure 3 in reference [2]. As a result, the same arachidonic acid concentration of 3.0 g/L was obtained. The results of this experiment suggests that the 24,25-methylenecholest-5-en-3β-ol compositional ratio in the culture of Figure 3 in reference [2] was as high as that obtained in this Experiment No. 2.

Table 2. Culture conditions and results of Experiment 2

Culture Conditions		
Experiment	Experiment No. 2	Reference [2]**
Fermentor scale	10-L fermentor	2000-L fermentor
Cultivation time	7 days	10 days
Major Medium Composition	2% glucose 1% yeast extract	2% glucose 1% yeast extract
Results		
arachidonic acid produced (g/L)	3.0 g/L	3.0 g/L
24,25-methylenecholest-5-en-3β-ol compositional ratio (%) (=A)	58.8	Not analyzed
Desmosterol compositional ratio (%) (=B)	23.0	Not analyzed
A/B	2.56	Not analyzed

**Cultivation of Figure 3 in reference [2] (Shinmen et al, 1989)

7. Reference 2 describes culturing of *Mortierella* in a 200-L fermentor with aeration. Reference 2 does not describe the ratio of 24,25-methylenecholest-5-en-3β-ol obtained. In Experiment 2, the method of Reference 2 was repeated using a 10-L fermentor. Since the productivity of arachidonic acid is the same between Reference 2 and Experiment 2, it is believed that the result of Reference 2 was reproduced in Experiment 2.

8. It is my opinion that the ratio of 24,25-methylenecholest-5-en-3β-ol obtained by the process disclosed in Reference 2 was likewise high. For Experiment 2, the same

strain of microorganism and same conditions as disclosed in Figure 3 of Shinmen et al were used, except a 10-L fermentor was used instead of a 2000-L fermentor and the cultivation time was 7 days rather than 10 days. Shinmen et al did not analyze the 24,25-methylenecholest-5-en-3β-ol compositional ratio as they did not recognize that 24,25-methylenecholest-5-en-3β-ol was even present. However, since the same amount of arachidonic acid was produced, it can be assumed that the compositional ratio of 24,25-methylenecholest-5-en-3β-ol would be the same.

9. The ratio of 2.56 would be outside the claimed range and would be significantly higher than applicants' instant invention.

10. I further declare that I am aware that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and my jeopardize the validity of any patent application or any patent issuing thereon. All statements made of my own knowledge are true, and all statements made on information and belief are believed to be true.

April 28 , 2003
Date

K. Higashiyama
Kenichi Higashiyama